

canonical nucleotide in place of at least a portion of the canonical nucleotides. The method comprises the steps of incubating a template nucleic acid in a reaction mixture containing a mutant nucleic acid polymerase which has

5 reduced discrimination between canonical and non-canonical nucleoside triphosphates, including between dNTPs and rNTPs, and the appropriate canonical or non-canonical nucleoside triphosphates which are substrates for the nucleic acid polymerase. One then follows standard polymerase reaction

10 protocols and creates the synthesized nucleic acid molecule.

Preferably, the reactions also contain inorganic pyrophosphatase, which is known to increase the yields in' *in vitro* transcription reactions (Cunningham, P.R. and Ofengand, J., 1990) and to reduce pyrophosphorolysis in

15 *in vitro* DNA synthesis reactions (Tabor, S., and Richardson, C.C., 1990), as well as buffers and other components which are known to those of skill in the art to be optimal for the particular w.t. polymerase used. Cunningham and Ofengand (1990) provide an example of conditions which may be used

20 for unprimed synthesis with T7 RNA polymerase or mutant T7 RNAPs, although one of skill in the art will recognize, with respect to reactions with these enzymes or other enzymes, the need to optimize the concentrations and ratios of canonical and non-canonical NTP substrates according to the

25 respective K_m and application and to modify reaction conditions, such as temperature, amount of enzyme, salt concentration, or divalent cation (e.g., Mg²⁺ or Mn²⁺) concentration, in order to produce improved results such as higher yield or a greater percentage of full-length

30 products.

In a preferred form of this method, the resulting synthesized nucleic acid molecule has a different

susceptibility to a nuclease compared to a nucleic acid synthesized by the corresponding non-mutant nucleic acid polymerase under identical reaction conditions with canonical substrates. By "different susceptibility" we mean 5 to include reduced, increased, or, in the case of synthetic nucleic acids containing both canonical and non-canonical nucleotides, altered susceptibility to a nuclease, which may be either a DNase or RNase. The nature of the reduced, increased or altered susceptibility to a nuclease is also 10 related to the properties of the nuclease. For example, a nucleic acid resistant to RNase A, which cleaves RNA only after C or U, may be synthesized using fewer non-canonical nucleotides (e.g., dNTPs or 2'-F-NTPs) than a nucleic acid which is resistant to RNase I, which cleaves after every 15 base.

In a preferred form of the present invention, the resulting synthesized nucleic acid is a ribozyme or a nucleic acid molecule used for gene therapy, in a vaccine as an antiviral composition, in an antimicrobial composition, 20 as an antisense composition for regulating gene expression, in a composition for hybridization to a complementary nucleic acid, such as for a primer, or as a probe for detection of a complementary nucleic acid.

The resulting synthesized nucleic acid may be either 25 single- or double-stranded.

The present invention is also a kit for performing the method of synthesizing a nucleic acid containing at least one non-canonical nucleotide. Typically, the kit contains a mutant nucleic acid polymerase with a reduced discrimination 30 for non-canonical compared to canonical substrates and data or information describing conditions under which the method may be performed.

The present invention is also improved methods for sequencing nucleic acids using a mutant nucleic acid polymerase of the present invention.

Because 2',3'-dideoxynucleotides are not substrates for wild-type RNA polymerase, it previously has not been possible to use the Sanger method for determining the sequence of a nucleic acid with an RNA polymerase, although 3'-deoxy- or 3'-hydroxymethyl analogs have been used as terminators for Sanger-like sequencing with RNA polymerases.

However, 2',3'-ddNTPs are substrates for the mutant nucleic acid polymerases of this invention which can also utilize both rNTPs and dNTPs as substrates, and the present invention is also a method for sequencing nucleic acid molecules (DNA or RNA) using a mutant nucleic acid polymerase and 2',3'-ddNTPs as terminators.

In one embodiment of this method, the nucleic acid to be sequenced, whether DNA or RNA, is used as a template for *in vitro* nucleic acid synthesis from a primer (i.e., primed synthesis) using a mutant RNA polymerase which has a reduced discrimination for dNTPs compared to rNTPs. Each of four different reactions also contains an amount of at least one nucleoside triphosphate corresponding to each nucleic acid base represented in either DNA or RNA, chosen from among the 2'-deoxynucleotides dATP, dCTP, dGTP and dTTP or dUTP, or the four common ribonucleotides ATP, CTP, GTP and UTP, or the 2'-fluorine-substituted nucleotides 2'-F-ATP, 2'-F-CTP, 2'-F-GTP and 2'-F-UTP or 2'-F-TTP. A 2',3'-dideoxynucleotide is also included in each *in vitro* nucleic acid synthesis reaction in an amount that will result in random substitution by the dideoxynucleotide of a small percentage of the corresponding rNTP, dNTP or 2'-F-NTP that is present